Single intramuscular dose toxicokinetics of manganese in broiler chicks

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Abstract

Aim: Manganese (Mn) produces neurobehavioral toxicity in various animal species including the young chicks. The present study examines toxicokinetics of Mn in 7-10-day old chicks after an intramuscular injection at 20 mg/kg.

Materials and Methods: Samples of the blood, whole brain, liver and kidney were obtained from chicks (5/each time period) at 0 time (base-line) and then at times between 0.17 to 4 h. The concentrations of Mn in the plasma and tissues were determined by atomic absorption spectrometry. Toxicokinetic parameters of Mn were calculated from the mean metal concentrations in the plasma by a non-compartmental analysis using a Windows-based computer program.

Results: Injection of Mn significantly increased the metal levels in the plasma, whole brain, liver and kidney of the chicks when compared to respective base-line control values at times 0.17 to 4 h after the injection (with the exception at 2 and 4 h in the brain). The highest concentration of Mn in the plasma and the whole brain appeared one h after the injection, whereas those of the liver and kidney appeared 4 h post-injection. The concentrations of Mn in the plasma ranged between 0.43 to 1.2 µg/ml within 0.17 to 4 h. Those of the whole brain, liver and kidney were 0.11– 0.46, 6.3–15 and 5.3–22.9 µg/g, respectively. The elimination half-life of Mn was 3.02 h with steady state volume of distribution 24.34 L/kg and total body clearances of 4.78 L/h/kg. The mean residence time of Mn was 5.09 h and its area under the plasma concentration-time curve (0-∞) was 4.18 µg.h/ml. The elimination half-life of Mn from the brain was 3.12 h with an elimination rate constant of 0.22 h-1.

Conclusion: The data suggest that Mn is well absorbed and rapidly distributed after an intramuscular administration in chicks and further support the reported neurobehavioral toxic effects of the metal which are observed within one h after treatment.

Key words: Chick; Half-life; Manganese; Neurotoxicity; Toxicokinetics.

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Introduction

Manganese (Mn) is known to be a neurotoxicant because of high industrial exposure level in man [1–3]. The neurotoxicity of Mn has been reproduced and characterized in various animal species including mainly rats and mice [4–8] with few studies in the avian species [9, 10]. The neurobehavioral effects of Mn in man and animals include disturbances of locomotor activity and alteration of cognitive behaviors [3, 8, 11-13]. A recent study also described a potential neurotoxicity model of Mn in broiler chicks [10].

The toxicokinetic and pharmacokinetic aspects of Mn and its tissue distribution are critical points for evaluating the toxicity and potential harmful effects of the metal [14–17]. Such information are important in the risk assessment of the metal [17–19]. The toxic effects of Mn might be associated with the differential accumulation of the metal in different organ systems of the body [16, 18]. For example, the neurochemical and neurobehavioral changes induced by Mn could be correlated with the differential accumulation of the metal in different regions of the brain [6, 20]. Plasma, bone and tissue levels of Mn are additionally used as diagnostic or biomarker endpoints of exposure [21–23].

The kinetic behavior of Mn has been described in several animal species with wide range of tissue distributions [14, 16, 18, 21]. In chicks, Mn administration at 10 to 100 mg/kg, intramuscularly (i.m.) produced high levels of the metal in the plasma, brain, liver and kidney [10]. Further, Mn administrations at 5, 10 and 20 mg/kg, i.m. were found to alter the locomotor activity and other behavioral performance in 7–14 days old broiler chicks [10]. However, the

Table-1. Manganese concentrations in the plasma (µg/ml) and tissues (µg/g) of chicks after a single intramuscular administration at a dose of 20 mg/kg body weight

Time (h)	Plasma	Brain	Liver	Kidney
0	0.10 ± 0.05*	0.11 ± 0.02*	0.58 ± 0.05*	0.44 ± 0.05*
0.17	0.43 ± 0.23	0.35 ± 0.05	10.3 ± 2.3	5.3 ± 1.2
0.33	0.49 ± 0.23	0.27 ± 0.03	7.8 ± 2.5	7.2 ± 2.1
0.50	0.66 ± 0.26	0.37 ± 0.02	10.5 ± 2.1	10.5 ± 2.4
0.75	0.69 ± 0.16	0.38 ± 0.04	7.7 ± 2.3	12.0 ± 4.2
1.0	1.20 ± 0.73	0.46 ± 0.05	13.3 ± 1.7	12.2 ± 5.4
1.5	0.88 ± 0.09	0.17 ± 0.03	6.3 ± 2.5	8.4 ± 4.7
2.0	0.49 ± 0.03	0.15 ± 0.02[a]	11.2 ± 2.5	15.8 ± 5.3
4.0	0.54 ± 0.36	0.11 ± 0.02[a]	15 ± 1.1	22.9 ± 2.1

Values are mean ± SE of 5 chicks/each sampling time. *Significantly different (except those with the superscript letter a) from respective concentrations between 0.17 to 4 h after the Mn injection, $p < 0.05$.

toxicokinetics of Mn are not known in chicks which could be used as an animal model of neurotoxicity induced by the metal. The purpose of the present study was to examine toxicokinetics of Mn administered at 20 mg/kg, i.m. in broiler young chicks.

Materials and Methods

One-day-old broiler chicks of both sexes obtained from a local hatchery were housed at room temperature of 32°C to 35°C with constant lighting, and floor litter consisted of wood shavings. Water and feed were available ad libitum. We performed the toxicokinetic experiment on 45 chicks when they became 7–10 days old. The Scientific Committee of the College of Veterinary Medicine at the University of Mosul has reviewed and approved the protocol of the study in chicks. All the experiments complied with institutional regulations addressing animal use, and proper attention and care were given to the chicks used in this study.

We prepared the injectable solution of Mn at 20 mg/kg by dissolving Mn chloride ($MnCl_2.4H_2O$, Avishkar, India) in unionized distilled water. The volume of administration was at 5 ml/kg, i.m. The choice of this dosage was based on our previous study in which Mn was found to induce behavioral changes in chicks [10]. Blood samples (1–2 ml) were collected from chicks (5/each sampling time) by jugular vein bleeding into heparinized test tubes [24] at 0 time (base-line) and then at times of 0.17, 0.33, 0.50, 0.75, 1, 1.50, 2 and 4 h after Mn administration. Thereafter, the chicks were euthanized by cervical dislocation to obtain the whole brain, liver and kidneys. Plasma was separated from erythrocytes by centrifugation of blood samples at 3000 rpm (Centurion, U.K.) for 15 minutes. Plasma and tissue samples were stored at –18ºC pending Mn determination within 48 h. The plasma and tissue samples were digested in 65% nitric acid with 24 h incubation at 70ºC [25]. The concentration of Mn was determined using atomic absorption spectrometry (Novaa 350, Germany) with UV-visible lamp and air-acetylene burner.

To reduce individual variations in the plasma concentrations of Mn, means of plasma concentrations of Mn at each sampling time (0.17–4 h) were used to calculate the toxicokinetic parameters by a non-compartmental analysis [26,27] using a Windows-based computer program [28]. The toxicokinetic variables included in the calculations were: area under plasma concentration-time curve ($AUC_{0-\infty}$), area under the moment curve ($AUMC_{0-\infty}$) from time zero to infinity, elimination half-life ($t_{1/2\beta}$), elimination rate constant ($k_{el}=0.693/t_{1/2\beta}$), steady state volume of distribution [V_{ss}=Dose.AUMC/ $(AUC)^2$], maximum Mn concentration (Cmax), time to maximum Mn concentration (Tmax), mean residence time (MRT=AUMC/AUC) and total clearance (CL= Dose/AUC). Furthermore, using a semi log paper, the $t_{1/2\beta}$ and k_{el} of brain Mn were calculated from Mn concentrations in the brain starting from Tmax at one h and then including the rest of the values until 4 h after the injection.

The differences of Mn concentrations in the plasma and tissues were statistically analyzed by analysis of variance followed by the least significant difference test [29]. The level of significance was at $p < 0.05$.

Results

Injection of Mn at the dose rate of 20 mg/kg, i.m. significantly and variably increased the metal levels in the plasma, whole brain, liver and kidney of the chicks when compared to respective base-line (0 time) control values at times 0.17 to 4 h after the injection (with the exception at 2 and 4 h in the brain), (Table 1). The highest concentration of Mn in the plasma and the whole brain appeared one h after the injection,

Table-2. Toxicokinetic parameters of manganese in chicks after a single intramuscular administration at a dose of 20 mg/kg body weight

Variable*	Unit	Value
Mean residence time (MRT=AUMC/AUC)	h	5.09
Steady state volume of distribution [Vss=Dose.AUMC/ $(AUC)^2$]	L/kg	24.34
Elimination rate constant (k_{el}=0.693/$_{t1/2\beta}$)	h^{-1}	0.23
Elimination half-life ($t_{1/2\beta}$)	h	3.02
Tmax	h	1
Cmax	µg/ml	1.2
Total clearance (CL=Dose/AUC)	L/h/kg	4.78
Area under plasma concentration-time curve ($AUC_{0-\infty}$)	µg/h/ml	4.18
Area under the moment curve ($AUMC_{0-\infty}$)	µg/h^2/ml	21.30

* The means of plasma concentrations of manganese at each sampling time (0.17–4 h) were used to calculate the toxicokinetic parameters by a non-compartmental analysis using a Windows-based computer program [28]. n=5 chicks/each sampling time.

whereas those of the liver and kidney appeared 4 h post-injection (Table 1). The concentrations of Mn in the plasma ranged between 0.43 to 1.2 µg/ml within 0.17 to 4 h (Table 1). Those of the whole brain, liver and kidney were 0.11–0.46, 6.3–15 and 5.3–22.9 µg/g, respectively (Table 1).

The toxicokinetic parameters of Mn calculated from the mean Mn concentrations in the plasma at times 0.17 to 4 h in chicks are shown in Table 2. The elimination half-life of Mn was 3.02 h with steady state volume of distribution 24.34 L/kg and total body clearance of 4.78 L/h/kg. The mean residence time of Mn was 5.09 h and its area under the plasma concentration-time curve (0-alpha) was 4.18 µg.h/ml. Other related toxicokinetic parameters are also listed in table 2. The elimination half-life of Mn from the brain was 3.12 h with an elimination rate constant of 0.22 h^{-1}.

Discussion

Manganese is widely distributed into various organs of the body and the metal burden correlates with toxic effects seen in the tissues [14–18, 21]. High levels of Mn are also attained in the plasma, brain, liver and kidney of chicks following the injection of the metal at doses ranged between 10-100 mg/kg, i.m. [10]. The findings of the present study are the first systemic toxicokinetic report of Mn in young chicks following its injection at 20 mg/kg, i.m. This dosage of Mn was reported to alter general locomotor activity of the chicks with indications of central depressant action [10]. The appearance of Mn in the plasma and the tissues especially in the brain within one h correlates with the behavioral changes reported in chicks within one h too [10]. The Cmax and Tmax values indicate that Mn is relatively rapidly absorbed into the systemic circulation of the chicks. High concentrations of Mn occurred in the kidney followed by the liver and the brain. In rats, Mn highly accumulates in the liver followed by the kidney and the heart at 2 h following an intravenous injection of $MnCl_2$ [30]. Appearance of Mn in the liver and kidney at high concentrations reflects the metabolic and excretory pathways of the metal within 4 h [14,30,31]. The pattern of Mn accumulation in the brain which is the target organ for the induction of neurobehavioral changes, depends on the chemical formulation of the metal and its route of administration [6, 20]. Mn readily enters the brain of younger animals compared to adults [32–34]. This favors the use of young chicks for monitoring the neurotoxic effect of Mn. Mn enters the brain through blood capillaries or by influx into the cerebrospinal fluid and across the choroid plexus [35, 36]. Injection of radioactive Mn into the blood circulation results within one h in the accumulation of the metal in the choroid plexus [37]. Generally, increased plasma and tissue Mn levels are considered as biomarkers of the metal exposure [21–23].

The toxicokinetic parameters of the present study suggest that Mn is well absorbed and distributed in the body of the chicks with a V_{ss} of 24.34 L/kg and eliminated relatively within a day (CL 4.78 L/h/kg and $t_{1/2}$ 3.02). V_{ss} is a reliable estimate of volume of distribution, since it is calculated independent of the k_{el} [26,27]. Increased levels of tissue Mn burden after systemic administration are in support of its high volume of distribution [14,16,18,21,30]. However, Mn is almost completely eliminated from the body within 5 days in rats [14,30,31]. The reported elimination half-life of Mn in rats is 4.56 h [14]. In our study it was 3.02 h. It should be expected that differences in the toxicokinetics of Mn would exist among various laboratory animal species. The variations in the kinetic parameters of Mn across various animal species could be attributed to the differences in the dosage and its form, chemical formulation, route of administration and species variation [6,14,16,18,20,

21,30,31]. The $t_{1/2\beta}$ of Mn in the brain was almost identical to that of the plasma (3.12 vs. 3.07 h). It was reported that Mn is slowly eliminated from the brain once it is accumulated there [32–34]. We did not observe this phenomenon in our current experiment. This could be attributed to the young age of the chicks used (7–10 days) as Mn is retained to a considerable extent in the brain of the adult animal only [20, 32–34]. The data suggest that Mn is well absorbed and rapidly distributed after an i.m. administration in chicks and further support the reported neurobehavioral toxic effects of the metal which are observed within one h after treatment.

Author's contribution

MAZ executed the experiments, shared in statistical analysis and shared in drafting the manuscript. FKM conceptualized the study, designed the experiment, supervised dosing regimen as well as data analysis, shared in statistical analysis and drafted the manuscript in English. All authors read and approved the final manuscript.

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Competing interest

Authors declares that they have no competing interest.

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